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High Susceptibility of Strain A Mice to Endotoxin and Endotoxin-Red Blood Cell Mixtures

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ABSTRACT

HEPPNER, GLORIA (University of California, Berkeley), AND DAVID W. WEISS. High susceptibility of strain A mice to endotoxin and endotoxin-red blood cell mixtures. J. Bacteriol. 90:696-703. 1965.—Strain A mice were shown to be considerably more susceptible to lethal effects of endotoxin lipopolysaccharide (LPS) than mice of several other strains. Complexes of sublethal quantities of LPS and sheep red blood cells were synergistically toxic for strain A mice. Separate administration of sheep red blood cells and heat-killed salmonellae, in either order and as long as 24 hr apart, also proved to be synergistically lethal for strain A mice, but not for R_{III} animals studied comparatively. Sheep red blood cell lysates possessed the ability of the intact cells in forming lethal combinations for strain A mice with killed salmonellae. Strain A red blood cell-killed salmonellae complexes were also lethal for strain A mice, but less so then complexes made with sheep red blood cells. A × R_{III} F_1 hybrid animals showed the same resistance characteristics as the resistant R_{III} parental strain. Possible explanations for these findings are suggested, and their relevance to an immunological mode of action of endotoxin lethality is discussed.

In the course of an investigation of the ability of mice of several inbred strains to make humoral antibodies to a mixture of sheep red blood cells (SRBC), bovine serum albumin, and heat-killed *Salmonella typhosa*, an unexpected observation was made. Although administration of this vaccine had no adverse effects on mice of the Cancer Research Genetics Laboratory (CRGL) strains R_{III}, C_3H, $C_3H/2$, $C_{57}Bl$, and Balb/c, strain A mice almost invariably died shortly after its injection. A further experiment showed that this lethal effect was produced only when the vaccine contained both SRBC and killed salmonellae. It appeared possible that this phenomenon was related to the earlier findings of Davis and Yull (1961) who, in attempting to reproduce experimentally the symptoms of shock following intestinal obstruction, detected a synergistic lethality between hemoglobin and living *Escherichia coli* in Sprague-Dawley rats (Davis and Yull, 1961). A study was undertaken to elucidate the present observation. Preliminary experiments suggested that strain A mice are highly susceptible to the lethal action of endotoxin and of complexes of sublethal quantities of killed salmonellae and SRBC formed in vitro, and that their red blood cells have a strong affinity for killed salmonellae and their endotoxin lipopolysaccharide (LPS; Hill and Weiss, 1964). The present communication reports the result of further investigation into the synergistic lethality of red blood cells and endotoxin for strain A mice.

MATERIALS AND METHODS

Animals. The following strains of mice were used: A/Crgl, R_{III}/Crgl, C_3H/Crgl, and $C_{57}Bl$/Crgl, obtained from the CRGL of the University of California, Berkeley; C_3H/HeJ, Jackson Memorial Laboratory; and Swiss albino mice.

The animals were fed a diet of water and pellets (Purina Lab. Chow), given *ad lib.*

Cultures. The strain of *S. typhosa* used was the ATCC strain 10243, kindly made available by A. Larson, Department of Bacteriology, University of California, Berkeley. Cultures were grown for 18 to 24 hr in Brain Heart Infusion (BHI) broth (BBL) at 37 C.

Preparation of heat-killed salmonellae. Quantities (5 ml) of BHI cultures were inoculated into 1,000-ml Roux bottles containing 400 ml of Trypticase Soy Agar (TSA, BBL). These were incubated for 18 hr at 37 C. The purity of the cultures was checked by microscopic examination of Gram-stained samples. The organisms were harvested from the TSA cultures by washing the growth off the agar with sterile saline. The organisms were killed by steaming the saline suspensions for 2 to 2.5 hr at 100 C. The steamed suspensions were then washed and resuspended in sterile saline at *p*H 7. These suspensions were stored at 4 C until used.

The number of organisms in the suspensions was determined turbidimetrically by reference to a standard curve relating optical density to microscopic count.

Before use in animals, the suspensions were tested for sterility by inoculating samples into tubes of thioglycolate broth, which were subsequently incubated at 37 C for 1 week and observed for growth.

In the experiments to be described, several preparations of heat-killed salmonellae, made at different times, were employed. In no way were these preparations observed to differ from one another.

LPS. The purified LPS used was the Difco product, no. 133138. Suspensions of LPS in saline at neutral *p*H were sterilized by steaming at 100 C for 30 to 60 min; this treatment also facilitates adsorption of the LPS onto red blood cells (Neter, 1956).

The method of Reed and Muench (1938) was used to perform LD_{50} determinations with the LPS.

Red blood cells (RBC). SRBC were obtained in Alsever's solution from Bennett Ranch and from Ansco Laboratory, Napa, Calif. Mouse RBC were collected by bleeding from an incision made with a razor blade across the ventral surface of the tail. Blood from several mice was pooled; the contribution of each individual mouse averaged about 0.5 ml. The blood was collected in Alsever's solution. The RBC were washed three times in barbital-buffered saline (*p*H 7.2) and packed by centrifugation at 1,000 × *g* before use. At no time were cells older than 1 week employed.

Preparation of RBC lysates. Packed SRBC were lysed by the addition of distilled water (1 ml/10 ml of SRBC). The stromata were removed by centrifugation at 1,200 × *g*. The lysate was diluted to the desired strength in buffered saline.

Preparation of RBC complexes for lethality assay. RBC–heat-killed salmonellae complexes were prepared by mixing 1 volume of washed, packed RBC with 3 volumes of heat-killed salmonellae suspended in sterile saline at a concentration of 2×10^{10} organisms per milliliter. This mixture was incubated at room temperature for 1 hr and then washed twice in barbital-buffered saline at *p*H 7.2 to remove free organisms. The complexes were then resuspended in the buffered saline to the desired concentration of red cells.

That an actual complex between RBC and whole salmonellae is formed by this method was evident from microscopic examination of salmonellae-treated RBC collected on a Millipore prefilter which allowed free bacteria to pass through the filter but retained the RBC. The cells were stained for 1 min with methylene blue. Bacterial bodies morphologically identical with the *Salmonella* culture were seen associated with the red-cell membranes. Moreover, RBC which had been exposed to killed salmonellae and subsequently washed were agglutinated by anti-*Salmonella* antiserum which had been previously absorbed with corresponding, untreated RBC.

RBC-LPS complexes were prepared by incubating a sample of a sterilized LPS suspension for 1 hr at 37 C with an equal volume of washed, packed SRBC. The treated RBC were then washed, packed, and resuspended in barbital-buffered saline to the desired concentration. Such RBC could also be agglutinated by anti-*Salmonella* antiserum.

TABLE 1. *Lethality of Salmonella LPS for various strains of mice*

Strain	Age	Sex	LPS LD_{50}*
	weeks		*μg*
R_{III}/Crgl	10	Female	707
		Male	>1,000
C_3H/HeJ	10	Male	>1,000
C_3H/Crgl	13	Male	500
$C_{57}B1$/Crgl	13	Male	410
Swiss	10	Male	392
A/Crgl	10	Female	177
		Male	202

* Calculated by the method of Reed and Muench (1938).

RESULTS

High susceptibility of strain A mice to the lethal action of endotoxin LPS. The initial observation which led to these experiments pointed to an unusual susceptibility of strain A mice to mixtures of SRBC and killed salmonellae. Subsequent experiments revealed strain A mice to be more susceptible to killed salmonellae and LPS alone than were strain R_{III} animals, arbitrarily chosen for purposes of comparison (Hill and Weiss, 1964). An experiment was conducted to extend this comparison to a number of different strains.

Saline dilutions of LPS ranging from 31 to 1,000 μg were injected intraperitoneally in 0.5-ml volumes per mouse into groups of 5 to 10 animals of the strains indicated in Table 1. The animals were observed until no further deaths occurred. The LD_{50} determinations (Table 1) show that mice of the A strain are more susceptible to LPS than those of any other strain tested. This observation suggested that the pronounced lethality of SRBC-salmonellae mixtures for strain A mice may have arisen from a potentiation of endotoxin lethality, brought about by action of the RBC. A study was therefore undertaken to define further the behavior of this lethal combination.

Synergistic lethality of SRBC and killed salmonellae. Experiments were carried out to determine whether SRBC and sublethal amounts of killed salmonellae must be given *simultaneously*

TABLE 2. *Lethality of SRBC and heat-killed salmonellae given separately at varying intervals to strain A females**

Substance injected (in 0.5 ml, ip)		Time between injections	No. of surviving animals†
First injection	Second injection		
		hr	
3×10^9 organisms‡	60% SRBC	0.5	0/5
60% SRBC	3×10^9 organisms	0.5	0/5
3×10^9 organisms	60% SRBC	1	0/5
60% SRBC	3×10^9 organisms	1	0/5
3×10^9 organisms	60% SRBC	2	0/5
60% SRBC	3×10^9 organisms	2	0/5
3×10^9 organisms	60% SRBC	4	0/5
60% SRBC	3×10^9 organisms	4	0/5
3×10^9 organisms	60% SRBC	8	1/5
60% SRBC	3×10^9 organisms	8	0/5
3×10^9 organisms	60% SRBC	12	1/5
60% SRBC	3×10^9 organisms	12	0/5
3×10^9 organisms	60% SRBC	24	0/5
60% SRBC	3×10^9 organisms	24	1/5

* See text for details.
† Death occurred 8 to 30 hr after the second injection.
‡ Heat-killed salmonellae.

to elicit the lethal effect, or whether they could be administered separately.

Thirty-five 9-week-old strain A females were given a single intraperitoneal (ip) injection of 3×10^9 heat-killed salmonellae in 0.5 ml of saline. This quantity of killed salmonellae is considerably below the lethal threshold when given alone (Hill and Weiss, 1964). At intervals of 0.5, 1, 2, 4, 8, 12, and 24 hr after this injection, groups of five mice each received a second ip injection of 0.5 ml of a 60% suspension of SRBC in buffered saline. Another group of 35 mice was first given the RBC suspension and, after the same time intervals, the killed salmonellae. A similar experiment was also carried out in 8- to 11-week-old A/Crgl males, with doses of 1.5×10^9 salmonellae and 40% suspensions of SRBC.

Separate ip administration of SRBC and sublethal quantities of heat-killed salmonellae, given in either order and as long as 24 hr apart, was lethal for strain A females (Table 2). The males, receiving fewer organisms and red cells, behaved similarly when the interval between injections was 30 min (Table 3). At greater intervals, however, many of the male animals survived.

The animals died 8 to 30 hr after injection of the second component of the mixture. Autopsy did not reveal a definitive pathology. Histological examination revealed numerous infarcts of the liver, with coagulation necrosis and a generalized infiltration of leukocytes. A large amount of hemosiderin was deposited around the large portal veins. The spleen also contained dense hemosiderin, and showed a marked increase of red pulp. The pathognomonic picture on the whole resembled that usually associated with shock.

Synergistic lethality of SRBC-LPS complexes. The high susceptibility of strain A mice to LPS as well as to heat-killed salmonellae, and the synergistic lethality of SRBC-salmonellae complexes (Hill and Weiss, 1964), suggested that these animals might also be very susceptible to complexes of SRBC and sublethal amounts of LPS.

Strain A males (20 weeks old) were injected ip with 0.5-ml quantities of suspensions of SRBC-LPS complexes. The RBC concentration of the complexes varied from 7.5 to 60%; the amount of LPS in the complexes was not determined, but it was no greater than 15 μg, since the total amount of LPS exposed to the SRBC was only 30 μg/ml of SRBC suspension.

Injection of the SRBC-LPS complex caused death of some of the strain A mice (Table 4). Moreover, all the surviving animals exhibited marked symptoms of toxicity: rapid breathing, ruffled fur, and diarrhea. SRBC-LPS complexes are thus seen to exert an effect similar to that of the SRBC-salmonellae complexes.

Synergistic lethality of SRBC lysate and heat-killed salmonellae. Experiments were conducted to determine whether the synergistic lethality of SRBC-killed salmonellae complexes depends on

TABLE 3. *Lethality of SRBC and heat-killed salmonellae given separately at varying intervals to strain A males**

Substance injected (in 0.5 ml, ip)		Time between injections	No. of surviving animals†
First injection	Second injection		
		hr	
1.5×10^9 organisms‡	40% SRBC	0.5	0/5
40% SRBC	1.5×10^9 organisms	0.5	0/5
1.5×10^9 organisms	40% SRBC	1	2/5
40% SRBC	1.5×10^9 organisms	1	4/5
1.5×10^9 organisms	40% SRBC	2	4/5
40% SRBC	1.5×10^9 organisms	2	2/5
1.5×10^9 organisms	40% SRBC	4	3/5
40% SRBC	1.5×10^9 organisms	4	2/5
1.5×10^9 organisms	40% SRBC	8	3/5
40% SRBC	1.5×10^9 organisms	8	0/5
1.5×10^9 organisms	40% SRBC	12	4/5
40% SRBC	1.5×10^9 organisms	12	4/5
1.5×10^9 organisms	40% SRBC	24	3/4
40% SRBC	1.5×10^9 organisms	24	4/5

* See text for details.
† Death occurred 8 to 30 hr after the second injection.
‡ Heat-killed salmonellae.

TABLE 4. *Effect of complexes of SRBC and Salmonella LPS for strain A mice*

Substance injected (in 0.5 ml, ip)	No. of surviving animals*
SRBC-LPS complex, 60% suspension	2/5
SRBC-LPS complex, 7.5 to 30% suspensions	15/15

* All the animals exhibited symptoms associated with endotoxin action (see text).

the morphological integrity of the SRBC, or whether it could be produced with SRBC lysates.

In a preliminary experiment, a group of 10-week-old strain A females were injected ip with 0.5 ml of a suspension containing 3×10^9 heat-killed salmonellae in 50% SRBC lysate. A second group of animals received the lysate suspension only.

All 10 strain A mice receiving the SRBC lysate together with the sublethal quantity of heat-killed salmonellae succumbed. No deaths occurred among mice injected with lysate alone. It appeared, therefore, that the lysate of SRBC could duplicate the ability of the intact SRBC to produce lethal effects in combination with killed salmonellae.

A more extensive experiment was then carried out in which 11-week-old strain A females and 10- to 13-week-old strain R_{III} males were injected ip with sublethal quantities of heat-killed salmonellae suspended in 0.5 ml of 50% SRBC lysate. The amount of killed salmonellae ranged from 8×10^7 to 3×10^9 organisms.

TABLE 5. *Effect of SRBC lysate and sublethal amounts of heat-killed salmonellae on strain A and R_{III} mice*

Substance injected (in 0.5 ml, ip)	No. of surviving animals	
	A*	R_{III}†
SRBC lysate + 3×10^9 organisms‡	0/5	4/5
SRBC lysate + 1.5×10^9 organisms	0/5	5/5
SRBC lysate + 7.5×10^8 organisms	0/5	5/5
SRBC lysate + 3.8×10^8 organisms	1/5	5/5
SRBC lysate + 1.6×10^8 organisms	3/5	5/5
SRBC lysate + 8×10^7 organisms	5/5	5/5

* Females, 11 weeks old.
† Males, 10 to 13 weeks old.
‡ Heat-killed salmonellae.

SRBC lysate in combination with as few as 1.6×10^8 heat-killed salmonellae was lethal for some of the strain A mice (Table 5). [More than 6×10^9 killed bacteria are required to elicit a lethal effect in the absence of SRBC (Hill and Weiss, 1964).] The R_{III} animals again proved to be resistant. The comparison between the strain A and R_{III} mice is somewhat clouded in this

TABLE 6. *Effect of SRBC lysate and sublethal amounts of heat-killed salmonellae on various strains of mice*

Substance injected (in 0.5 ml, ip)	No. of surviving animals of strains				
	R_{III}	C_3H/HeJ	$C_{57}Bl$	Swiss	A
SRBC lysate + 2.5 × 10^9 organisms*	5/5	5/5	2/5	5/5	0/5
SRBC lysate + 1.3 × 10^9 organisms	5/5	6/6	5/5	5/5	0/4
SRBC lysate + 5 × 10^8 organisms	5/5	5/5	5/5	5/5	2/5
SRBC lysate + 2.5 × 10^8 organisms	5/5	5/5	5/5	5/5	5/6
SRBC lysate + 1.3 × 10^8 organisms	5/5	5/5	5/5	5/5	5/5

* Heat-killed salmonellae.

TABLE 7. *Lethality for strain A mice of SRBC lysate and heat-killed salmonellae given separately at varying intervals*

No. of killed salmonellae injected (in 0.5 ml, ip)	Time interval between injection of SRBC lysate* and killed salmonellae	No. of surviving animals
	hr	
3 × 10^9	1	1/5
7.5 × 10^8	1	3/5
3 × 10^9	3	0/5
7.5 × 10^8	3	1/5
3 × 10^9	6	0/5
7.5 × 10^8	6	2/5
3 × 10^9	12	2/5
7.5 × 10^8	12	5/5
3 × 10^9	24	5/5
7.5 × 10^8	24	5/5

* The animals received an initial ip injection of 0.5 ml of a 50% SRBC lysate.

experiment by the sex difference; however, males and females were not found to differ markedly in LPS susceptibility (Table 1), and the sex difference probably does not account for the distinctive reactions of the two strains.

The above experiment was repeated with mice of strains C_3H/HeJ, $C_{57}Bl$/Crgl, and Swiss albino, as well as with the R_{III} and A. Groups of 10-week-old males of these strains were injected ip with quantities of salmonellae ranging from 1.3 × 10^8 to 2.5 × 10^9 suspended in 50% SRBC lysate.

Strain A mice were unique in their susceptibility to SRBC lysate and heat-killed salmonellae (Table 6). Only a few strain $C_{57}Bl$ mice died as a result of the lysate-salmonellae injections, and these deaths occurred in the group receiving the highest concentration of salmonellae. All other animals in this experiment, except those of strain A, survived.

An experiment was undertaken to ascertain whether separate administration of lysate and salmonellae would produce a synergistically lethal effect, as was the case with separate injections of SRBC and whole, killed salmonellae.

Fifty 4-month-old strain A males were injected ip with 0.5-ml quantities of 50% SRBC lysate. After 1, 3, 6, 12, and 24 hr, groups of five mice each were given a second injection of either 7.5 × 10^8 or 3 × 10^9 heat-killed salmonellae suspended in saline.

Injection of SRBC lysate and killed salmonellae, even at 12-hr intervals, still produced synergistic lethality for strain A mice (Table 7). Experiments are in progress to test whether SRBC stromata can also potentiate the lethality of killed salmonellae.

Synergistic lethality of strain A mouse RBC and heat-killed salmonellae. The demonstration of the high affinity of strain A mouse red cells (ARBC), as well as of sheep red cells, for heat-killed salmonellae and LPS (Hill and Weiss, 1964) suggested that ARBC might also act synergistically with the killed bacteria to cause death in strain A mice.

Thirteen 4-month-old strain A males were injected ip with 0.5 ml of 50% ARBC-salmonellae complex suspension in saline. A control group of seven mice received ARBC alone, and a second control group of five mice were injected with SRBC-salmonellae complex.

Only 2 of the 13 strain A mice given the ARBC-salmonellae complex died, whereas 5 out of the 5 receiving SRBC-salmonellae complex succumbed (Table 8). The 50% suspension of untreated ARBC was innocuous. All animals injected with ARBC-salmonellae complex exhibited marked symptoms of toxicity. Although SRBC-salmonellae complexes appeared to be more toxic to strain A mice than ARBC complexes, the latter were capable of lethal action. Their lower toxic potency might be due to a lesser ability of ARBC to adsorb the bacteria; this explanation awaits quantitative study.

Effect of SRBC-salmonellae complexes on strain A × R_{III} hybrids. Experiments were initiated to analyze genetically the pronounced susceptibility of strain A mice to killed salmonellae, LPS, and RBC-salmonellae complexes. The results of the first completed experiment are described.

Strain A and R_{III} mice were mated to obtain F_1 progeny. Reciprocal matings were attempted, but pregnancy resulted only when the females

TABLE 8. *Lethality of complexes of strain A RBC and heat-killed salmonellae for strain A mice*

Treatment (in 0.5 ml, ip)	No. of surviving animals
Strain A RBC, 50% suspension	7/7
Strain A RBC adsorbed with salmonellae, 50% RBC suspension	11/13*
SRBC adsorbed with salmonellae, 50% RBC suspension	0/5

* All the animals exhibited symptoms associated with endotoxin action.

TABLE 9. *Effect of complexes of SRBC and killed salmonellae for strain A × R_{III} hybrid mice*

Mice injected with 60% suspension of SRBC-salmonellae complex (in 0.5 ml, ip)	No. of surviving animals
Strain A females, 12 weeks old	0/10
Strain R_{III} females, 8 weeks old	10/10
Strain (A × R_{III}) hybrid females, 3 to 6 months old	13/13
Strain (A × R_{III}) hybrid males, 3 to 6 months old	8/8

were of the A strain. The hybrid young were weaned at 1 month of age and separated on the basis of sex. When they were 3 to 6 months old, they were injected ip with 0.5 ml of a 60% SRBC-salmonellae complex. Strain A (12 weeks old) and R_{III} females (8 weeks old) were included in the experiment as controls.

All the strain A mice succumbed to the SRBC-salmonellae complex, whereas neither the R_{III} nor the (A × R_{III}) F_1 animals were lethally affected (Table 9). The F_1 hybrids displayed the resistance characteristics of the resistant parental strain.

DISCUSSION

The initial observation which suggested the present experiments was the death of strain A mice, but not of any other strain, after the injection of a mixture of SRBC and heat-killed salmonellae. The finding that LPS could be substituted for the killed bacilli in the synergistically lethal mixture points to endotoxin as the most likely cause of death. The demonstration that strain A mice are more susceptible to the lethal effects of LPS alone than are animals of any of several other strains tested gives added support to this hypothesis.

Death from endotoxin is not marked by definitive pathological characteristics. The general picture is that of shock (Thomas, 1954). It is not possible, therefore, to clearly diagnose endotoxin death on the basis of pathognomonic picture. The pathology of strain A mice which died after administration of salmonellae alone or of salmonellae together with SRBC also failed to reveal distinctive characteristics; shock was the diagnosis for the cause of death. The appearance of these animals before and at death is, thus, compatible with the incrimination of endotoxin as the lethal agent.

The mode of action of endotoxin lethality is not clear (Fine, 1964; Janoff and Kaley, 1964). It has recently been proposed by some investigators that endotoxin shock may be related to the phenomenon of delayed hypersensitivity (Stetson, 1959, 1964), but the proposal has been challenged by other workers (Stinebring, Axelrod, and Trakatellis, 1964; Heilman, 1964). An immunological etiology of endotoxin shock would provide an attractive conceptual framework for the present observations. Strain A mice have been reported to be highly reactive immunologically. Thus, it was shown that the level of circulating antibody after immunization with SRBC is higher in strain A mice than in mice of other strains (Davidsohn and Stern, 1954). Similarly, there is a stronger circulating antibody response to *Salmonella* 0 antigen in strain A than in strain R_{III} mice (*unpublished data*). Strain A mice also exhibit considerable immunological responsiveness to spontaneous mammary tumors (Attia and Weiss, *unpublished data*), and females are more resistant to male isografts than are females of strains Balb/c, C_3H, CBA, and DBA (Klein and Linder, 1961). If endotoxin death does, indeed, result from an immunological event, strain A mice could be expected to be especially susceptible among animals of this species.

Recent observations which point to the possession of immunological adjuvant properties by RBC also suggest an immunological explanation for the lethality of red cell-salmonellae or LPS complexes in the strain A mouse. It has been shown by Neter et al. (1964) that attachment to RBC greatly enhances the antigenicity of gram-negative "common antigen" for rabbits. The mechanism of RBC adjuvant action in that system is uncertain, but the suggestion was advanced that perhaps adsorption of the antigen onto the erythrocyte surface causes it to be more efficiently transported to sites of antibody formation. Neter et al. (1964) also suggested that perhaps "common antigen" alone is only a hapten, and must be adsorbed onto the cell surface to elicit antibody production.

The adjuvant activity of RBC could also reside in an internal component of the cell. The demonstration of the adjuvant action of ribonucleic acid (RNA; Merritt and Johnson, 1964) suggests

that RNA in RBC could play such a role. An adjuvant action by hemoglobin aggreggates has also been claimed (Nelson, 1957). The present findings that SRBC lysates have the activity of the intact cells in forming lethal combinations with killed salmonellae could thus be interpreted within a hypothesis ascribing adjuvant action to RBC and, hence, a potentiation of the immunopathological effects of endotoxin. [It should be noted that many immunological adjuvants enhance the delayed hypersensitivity component of immunological reactions in addition to, or instead of, furthering a humoral antibody response (White, 1963).]

Although an immunological interpretation of the present findings is attractive, it must be emphasized that there is no direct proof for such a theory. It can be imagined that RBC and their lysates may potentiate endotoxin lethality in a nonimmunological manner. Thus, for example, it is known that gelatin, which probably does not act as an immunological adjuvant, potentiates the lethal effects of botulinum toxin, a substance whose toxigenicity does not appear to stem from immunological mechanism (Gladstone, 1962). The possible action of intact RBC and of their lysates on the translocation of endotoxin in the body may conceivably enhance its toxicity by bringing it more effectively to susceptible sites.

It must also be pointed out that there is no evidence that RBC factors act unidirectionally to potentiate endotoxin. It is known that RBC lysates can exert toxic effects, especially on the kidney and liver (Forbus, 1952). It is not impossible, therefore, that endotoxin [a known adjuvant (Johnson, 1964)] somehow potentiates RBC lysate toxicity. Neither is it impossible that RBC and killed salmonellae or LPS form complexes possessing *new* toxic properties.

Studies are in progress to quantify and extend the present observations, in the hope of gaining further insight into (i) the unique susceptibility of strain A mice to killed, endotoxin-containing bacteria and to LPS, and (ii) possibly into the mechanisms of endotoxin lethality as such.

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